

## Reagents Provided

**Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human Neuropilin-1:** Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 446921

**Isotype:** mouse IgG<sub>2A</sub>

## Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

## Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

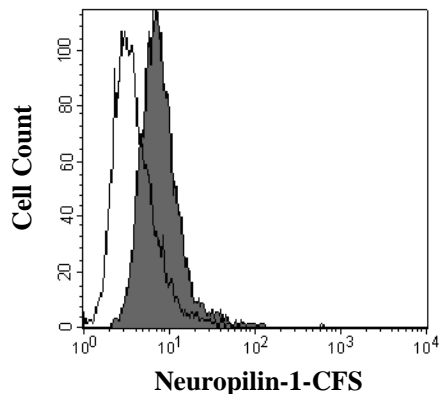
Designed to quantitatively determine the percentage of cells bearing Neuropilin-1 within a population and qualitatively determine the density of Neuropilin-1 on cell surfaces by flow cytometry.

## Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody, which binds to cells expressing Neuropilin-1. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing Neuropilin-1 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Neuropilin-1. Cell surface expression of Neuropilin-1 is determined by flow cytometry using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515 - 545 nm.

## Reagent Preparation

**Fluorescein-conjugated mouse anti-human Neuropilin-1:** Use as is; no preparation necessary.



HUVEC cells were stained with CFS-conjugated anti-human Neuropilin-1 (Catalog # FAB3870F, filled histogram) or isotype control (Catalog # IC003F, open histogram).

2009/05/22

## Sample Preparation

**Peripheral blood cells:** Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) followed by centrifugation at 500 x g for 5 minutes. 50 µL of packed cells should then be transferred to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

**Cell Cultures:** Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA) to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10<sup>6</sup> cells/mL and 25 µL of cells (1 x 10<sup>5</sup>) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

## Sample Staining

- 1) Cells should be Fc-blocked by treatment with 1 µg of human IgG/10<sup>5</sup> cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
- 2) Transfer 25 µL of the Fc-blocked cells (1 x 10<sup>5</sup> cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of CFS-conjugated Neuropilin-1 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted Neuropilin-1 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
- 6) Finally, resuspend the cells in 200 - 400 µL of PBS buffer for analysis by flow cytometry.
- 7) As a control for this analysis, cells in a separate tube should be treated with CFS-labeled mouse IgG<sub>2A</sub> antibody.

This procedure may need modification, depending upon final utilization.

**Background Information**

Neuropilin-1 is a type I transmembrane protein that is expressed in the developing nervous system and by endothelial and tumor cells. Neuropilin-1 binds members of the class III secreted semaphorin subfamily as well as some isoforms of VEGF family proteins.

**Warning:** Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.